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Environmental filtering vs. resource-based niche partitioning in diverse soil animal assemblages

Running title: Niche in soil communities

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Abstract

Terrestrial invertebrates constitute most of described animal biodiversity and soil is a major reservoir of this diversity. In the classical attempt to understand the processes supporting biodiversity, ecologists are currently seeking to unravel the differential roles of environmental filtering and competition for resources in niche partitioning processes: these processes are in principle distinct although they may act simultaneously, interact at multiple spatial and temporal scales, and are often confounded in studies of soil communities. We used a novel combination of methods based on stable isotopes and trait analysis to resolve these processes in diverse oribatid mite assemblages at spatial scales at which competition for resources could in principle be a major driver. We also used a null model approach based on a general neutral model of beta diversity. A large and significant fraction of community variation was explainable in terms of linear and periodic spatial structures in the distribution of organic C, N and soil structure: species were clearly arranged along an environmental, spatially structured gradient. However, competition related trait differences did not map onto the distances separating species along the environmental gradient and neutral models provided a satisfying approximation of beta diversity patterns. The results represent the first robust evidence that in very diverse soil arthropod assemblages resource-based niche partitioning plays a minor role while environmental filtering remains a fundamental driver of species distribution.

Keywords: stable isotopes, trophic niche, community structure, neutral theory, soil microarthropods, oribatid mites

1. Introduction

The classical view of communities and the assembly processes forming them has historically been dominated by the approaches pioneered by the founders of niche theories. More recently classical theories have been rethought to include stochastic processes such as those related to stochastic demographic fluctuations and dispersal dynamics, which for example are the only mechanisms postulated in neutral theories (Bell, 2001; Hubbell, 2001). Stochastic processes have also been included in the more general framework of metacommunity theories (Cottenie, 2005; Leibold et al., 2004), which focus on the spatial nature of assembly processes and extend the principles of metapopulation dynamics to community ecology. For example, processes such as dispersal create spatial patterns in species distribution. These spatial patterns do not depend on spatial structure in the distribution of environmental variables although the processes generating these patterns may interact with environmentally driven processes (Smith and Lundholm, 2010). Biotic interaction, too, can create spatial patterns (e.g., segregation of competing species in fairly homogeneous environments), regardless of other spatial processes (Gotelli, 2000; Gotelli et al., 2010). Environmental gradients determine spatial patterns in species distribution by sorting species according to their environmental requirements (e.g., dry-tolerant vs. moist tolerant species) and for a long time community ecology has been synonymous with studying species distributions along such gradients (Morin, 2011).

These various processes are entangled in nature at multiple spatial scales but a key general point we analyse in this paper is that environmental filtering is one component of niche partitioning dynamics, which might or might not involve resource based niche partitioning due to competition for shared resources (Adler et al., 2013; HilleRisLambers et al., 2012; Hubbell, 2005; Kraft et al., 2014). Interestingly, the point of possible independence of environmental filtering and resource-based niche

partitioning has been made both by niche (HilleRisLambers et al., 2012; Kraft et al., 2014) and neutral theorists (Hubbell, 2005) in spite of the fact that several ecologists in practice continue to see niches in the sense of Grinnell, that is to say in terms of species environmental requirements (Chase and Leibold, 2003).

Invertebrates constitute most of animal biodiversity and soil is a major reservoir of this diversity. Soil animal community ecologists, following other animal and plant ecologists (Dornelas et al., 2006; Hubbell, 2001; Ritchie, 2009), for a long time have addressed taxonomically defined assemblages such as oribatid mites, collembolans or nematodes to unravel the mechanisms that allow species coexistence in very diverse systems (Wardle, 2002). Recently, microarthropods have also been investigated within the niche-neutral debates or the more general framework of metacommunity theories (Caruso et al., 2012; Lindo and Winchester, 2009; Nielsen et al., 2010; Salmon and Ponge, 2012). However, in recent years studies based on stable isotopes and molecular genetics have clearly shown that assemblages such as oribatid mites or collembolans actually consist of species that can range in diet from being decomposers of low quality organic matter to being top predators of nematodes (Heidemann et al., 2011; Maraun et al., 2011; Schneider et al., 2004). This fact implies a strong bias of previous studies in terms of how observed patterns can inform on underlying mechanisms. For example, if we test neutral theories against niche partitioning theories, we should test these within trophic levels (Hubbell, 2005), which challenges previous studies (Caruso et al., 2012; Gao et al., 2014; Lindo and Winchester, 2009; Nielsen et al., 2010). In general, there is little theoretical and empirical support for the hypothesis that soil animal communities are structured by niche dynamics based on competition (Gao et al., 2014; Wardle, 2006), although several studies have shown that microarthropod communities are sorted by environmental gradients (Auclerc et al., 2009; Lindo and Winchester, 2009; Salmon and Ponge, 2012).

We addressed this general point by focusing on diverse soil oribatid mite assemblages from a dry grassland using a spatially explicit sampling design that allowed us minimise dispersal processes and focus on environmental filtering and niche partitioning based on food resources. Instead of focusing on taxonomic assemblages, we used the stable isotopes ratios $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$, and for the first time focus community analysis on trophic assemblages within which competition for shared resources could be a key process. To further characterise species in terms of traits that can be related to competition for resources, we quantified body size and depth distribution and then defined a trait matrix. We used these data to test the hypothesis that species that were closer in space and time were more dissimilar and vice-versa (limiting similarity concept) than expected by chance. The assumption is that limiting similarity and/or trait trade-offs should be observed if resource based niche partitioning is a mechanism through which species coexist locally while competing for shared resources. Still, resource-based niche partition and environmental filtering may act simultaneously. Thus, species could also be sorted along environmental gradients either in relation to the measured traits or not. In fact, environmental filtering and resource-based niche partition could also be decoupled if competition is not taking place or is of minor importance. The rationale behind the test of these hypotheses is that demonstrating a clear link between trait differences and environmental distance is a key premise to unravel the mechanisms that allow species coexistence in rich communities (Adler et al., 2013).

2. Materials and Methods

2.1 Study area and sampling strategy

This study was conducted in dry grassland in a natural reserve in Mallnow, Lebus, (Brandenburg, Germany, 52°27.778' N, 14°29.349' E). This reserve has been managed

by low-intensity sheep grazing for at least 500 years and is dominated by *Festuca brevipila* (Poaceae). There are areas where grazing may not occur for one year or longer and plant diversity can be very high locally (e.g., > 40 species in a 10 x 10 m plot) although grasses such as *Festuca* spp. dominate the assemblage. In these areas, in April and October 2012 we took soil core samples (local communities) within two undisturbed plots of 15 x 15 m along the slope of a hillside, with the two plots about 20 m apart. The two plots represented spatial replicates of a steep soil textural gradient running from the sandy-loamy soil uphill to highly sandy soil downhill. Main soil parameters such as pH, water content, organic C and N varied along the gradient, in some case with remarkable variation (Supplementary Material, Table S1). Sampling was replicated in the two main seasons (spring and autumn). To standardise the local soil arthropod community, we took soil cores (5 cm diameter, 10 cm deep) centred on the grass *Festuca brevipila*, which was by far the most abundant species in the area (in some case cover > 70%). Twenty randomly positioned samples per plot were collected in each season (total of 80 local communities) and the position of each sample was recorded in the UTM system.

2.2 Sample processing and analysis

Each soil core was cut into five 2 cm slices to quantify species depth distribution. However, the soil core was the main unit of analysis and we defined the local assemblage as the species inhabiting this unit. Eventually, each species was assigned a depth score based on the weighted average of its depth distribution and depth was treated as a species trait. The soil fauna was extracted in a Macfadyen apparatus for two weeks. All arthropods were preserved in 70% ethanol and the adult oribatids morphologically determined to species level (Weigmann, 2006). Body lengths were measured for each individual under a dissecting microscope (Leica M 165, Wetzlar, Germany) using the

software LAS. Each species was assigned a size score based on the average length obtained from a number of replicated measurements (mean number of measurements per species = 85; median number of measurements per species = 30). Soil water content was measured as the difference between the weights of fresh vs. dried soil (soil dry weight, SWD), with samples collected at field capacity. Soil pH was measured in a soil-water suspension, where 3 g of soil and 15 ml distilled H₂O were mixed and stirred. The measurement was conducted in the supernatant until the value remained constant. Organic carbon (C) and total nitrogen (N) were measured by direct combustion of 30 mg of soil in a Euro EA Element Analyzer (HEKAtech GmbH, Wegberg, Germany). Mean weight diameter (MWD) was calculated as the weighted sum of the proportion of soil particles and aggregates in each size class (2-4 mm, 1-2 mm, 0.5-1 mm and 0.2-0.5 mm), determined by dry sieving of the soil.

2.3 Stable isotope analysis

Specimens were transferred into tin capsules. Rare (e.g. *Carabodes willmanni*) or smaller-sized species (e.g. *Microppia minus*) required the pooling of several individuals to reach the biomass necessary to the analysis. After drying at 60°C for at least 12 h, samples were reweighed and stored in a desiccator until further analysis. The same procedure was used to prepare samples of nematodes, extracted from fresh soil by using a modified Baermann funnel method. Soil, mosses, lichens, roots, and plant material were ground and subjected to the same procedure (root and plant material 1.0 - 1.5 mg, soil 34.1 - 35.3 mg). We analysed these organisms and material to obtain baseline values of different potential food sources for oribatid mites (Supplementary Material). A coupled system of an elemental analyzer (Euro EA 3000, Euro Vector S.p.A.: Milano, Italy) and a mass spectrometer (Delta V Plus Thermo Electron; Bremen, Germany) was used to analyze the ¹³C/¹²C and ¹⁵N/¹⁴N ratios (Reineking et al., 1993). The primary standard for ¹⁵N was atmospheric

nitrogen whereas acetanilide (C_8H_9NO , Merck, Darmstadt, Germany) served for internal calibration. Vienna Pee Dee Belemnite (V-PDB) was used as a primary standard for ^{13}C . See also Fischer et al. (2010), Maraun et al. (2011), Pollierer et al. (2009), and Schneider et al. (2004) for further details.

2.4 Data analysis

We used stable isotopes to focus on a diverse but narrowly defined trophic assemblage. We based the definition of ‘relatively narrow trophic assemblage’ on the concentration of ^{15}N , which increases from food sources to consumers (Deniro and Epstein, 1981; Peterson and Fry, 1987; Scheu, 2002). The enrichment of ^{15}N varies with diet, especially in generalists, but despite this variation, an average enrichment of 3.4‰ is commonly used to define trophic groups (Post, 2002). The concentration of ^{13}C is usually associated with the analysis of ^{15}N because ^{13}C reflects the basal food source (Deniro and Epstein, 1981; Peterson and Fry, 1987; Post, 2002). The variance of stable isotope signatures reflects the dietary niche width of consumers (Bearhop et al., 2004), which led some authors to define the concept of isotopic niche (Newsome et al., 2007). Eventually (see results) we could define a set of 18 species that potentially competed for fungal resources, and we focused our analysis on this assemblage.

In order to visualise and quantitatively summarise the multivariate covariation of environmental variables (Organic C, N, C:N, Water, pH, Mean Weight Diameter of soil particles) and major gradients, we performed a Principal Component Analysis (PCA) on the correlation matrix of the variables (Legendre and Legendre 1998; Gotelli and Ellison 2004). We used PCA axes as environmental correlates of species distribution to eliminate collinearity in predictors (Gotelli and Ellison 2004).

Given the small scale of the study and all else being equal, we used a modelling strategy consisting of several steps to test the general hypothesis that species closer in space and

201 time were more dissimilar in terms of traits related to competition for resources
 202 (limiting similarity concept): if resource based niche partitioning is a mechanism
 203 through which species coexist locally while competing for shared resources, then
 204 limiting similarity or trait trade-offs should be observed (HilleRisLambers et al., 2012;
 205 Adler et al. 2013). In order to test this hypothesis, we first used a multivariate regression
 206 approach based on RDA (Borcard et al., 2004, 1992; Legendre and Legendre, 1998) to
 207 empirically define the spatial and temporal niches of each species. We Hellinger
 208 transformed raw data to meaningfully apply RDA, which is PCA-based (Euclidean
 209 space), and ensure no inflation of the weights of rare species (Legendre and Gallagher,
 210 2001). The spatially explicit and seasonal sampling design together with the
 211 measurement of several crucial environmental variables allowed us to model species
 212 distribution as a function of both spatial and environmental factors, and changes
 213 between the two sampled seasons. We used the well-established method of principal
 214 coordinate analysis of neighbour matrices (PCNM; Borcard and Legendre, 2002) to
 215 define a set of spatial factors that parsimoniously accounted for patterns in species
 216 distribution. The final set of PCNM vectors was defined using a multivariate extension
 217 of the Akaike information criterion (AIC; Dray et al., 2006). Environmental factors
 218 were soil water content (% dry weight), pH, organic C, total N, the C:N ratio, and the
 219 mean weight diameter of soil aggregates, used as a proxy for soil structure (Caruso et
 220 al., 2011). We used the species scores of the statistically significant axes of the RDA
 221 model to define species niches: by definition, the Euclidean distance between any two
 222 species in the vectorial space defined by RDA axes reflects predicted distances in space,
 223 seasons, and environmental conditions: the further apart any two species are in the RDA
 224 space the further apart these species are in space, time, and average environmental
 225 characteristics of the patches they colonise. We also used permutational tests to test for
 226 the effects of spatial and environmental factors, including partial effects (i.e. testing for

one factor while statistically controlling for other factors). Once we defined the RDA model-based spatial, temporal and environmental position of species (Grinnellian niche), we used body size and depth distribution together with the $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ signature to define a species trait matrix. After data standardization and calculation of Euclidean distance, a trait distance matrix of species was obtained. We finally used a Mantel test to test the hypothesis of a negative correlation between the trait distance matrix and the distance matrix based on space, season, and environment: we expected a negative correlation under the limiting similarity hypothesis because the more similar species are in traits involved in competition the more distant species should be in their Grinnellian niche. In practice, species minimise spatial and temporal coexistence to avoid competition and at the same time can coexist locally if they differ in key traits. Conversely, the closer species are in terms of spatial, temporal and environmental position the less similar they should be in terms of traits involved in competition. We used the R packages *vegan*, *spacemaker* and *ade4* for all multivariate analyses (Chessel et al., 2004.; Dray et al., 2006; Oksanen et al., 2009).

We completed our analysis with a neutral model, based on the null assumption that trophically similar species are not involved in resource-based niche partitioning when they come together to form assemblages. To fit a general neutral model, we used the formula for multiple samples and a PARI/GP code (Etienne, 2007) to estimate neutral model parameters θ (diversity) and I (immigration rate). Afterwards, we used the PARI/GP function *urn2.gp* (Etienne, 2007) to create 4999 neutral communities based on the estimate parameters. We applied this approach to the following datasets: all species across all trophic levels (spring and autumn, respectively), and just fungal feeders (spring and autumn, respectively). The simulated communities were used to build a null distribution of beta diversity values. We quantified beta diversity (BD) following Legendre and De Cáceres (2013): the sum of species variances in the species by site

matrix (with usual correction terms for unbiased estimates of variance). Data were Hellinger-transformed (Legendre and Gallagher 2001). The observed value of BD was compared to the null distribution: if observed BD was within the 95% interval of the simulated data sets, the neutral model could not be rejected at $p < 0.05$ (Maaß et al., 2014).

3. Results

3.1 Environmental variation

PCA of environmental variables (Fig. 1) summarised more than three quarters of total variation in the first two axes. Although all variables have some effect on all PCA axes, PC1 (53%) described a main gradient mostly due to organic matter (organic C and total N) and soil structure (Mean Weight Diameter, MWD) while PC2 (24%) mostly accounted for a negative covariation between water content and C:N ratio. Consistently with the construction of our sampling strategy, the gradients were maximised along the up- to down-hill direction, with some variation between the two sampling plots (Supplementary Material, Table S1): the gradient in organic matter and soil structure was more pronounced in Plot 1 while the negative correlation between water and C:N was more pronounced in Plot 2. There was no significant difference between spring and autumn samples for either plots (Supplementary Material, Fig. S1). Absolute variation in individual soil variables was remarkable in some case: for example, organic C content ranged from 0.15 to 3.49%, total N from 0.01 to 0.26%, and pH from 4.8 to 8.9, and these ranges were comparable between the two plots.

3.2 Oribatid mite assemblage and isotopes

In total, we collected 2,397 adult Oribatids of 33 species belonging to 18 families. The most abundant species in both seasons were *Liebstadia pannonica*, *Punctoribates*

punctum and *Peloptulus phaenotus*. There were five species (*Achipteria coleoptrata*,
Carabodes willmanni, *Trichoribates novus*, *Galumna obvia*, and *Minunthozetes*
semirufus) that were present with few individuals (1 to 4) only in one of the two
seasons. Rarefaction curves (not shown) confirmed that the sampling effort was
sufficient to describe the overall richness of the oribatid community. We obtained ^{15}N
and ^{13}C data for 28 species (Supplementary Material, Fig. S2 and Table S3). *Microppia*
minus and *Porobelba spinosa* showed the highest ^{15}N signatures whereas *Carabodes*
willmanni had the lowest ^{15}N signature. Three species (*M. semirufus*, *T. vel. sarekensis*,
S. sculptus) had very similar ^{15}N signatures comparable with the root signatures while
mosses, lichens, and nematodes were about one trophic level below their potential
consumers/predators (Supplementary Material, Fig. S2 and Table S1).
Overall, the stable isotope analysis and relevant literature (Fischer et al., 2010; Maraun
et al., 2011; Pollierer et al., 2009; Schneider et al., 2004) allowed us to group the
oribatid mite community into five trophic groups (predators, fungal feeders/secondary
decomposers, decomposers, lichen feeders and species with endophagous
juveniles/tunnelers, see Supplementary Material). However, for *T. novus*, *Passalozetes*
perforates and *M. semirufus*, the group affiliation was not clear. We consider *P.*
perforates to be a mycophagous species and *M. semirufus* a moss feeder but definitive
evidence is missing. The feeding preferences of *T. novus* remain unclear.
Based on these data, we defined a group of 18 species (Table 1; Supplementary
Material, Table S2) in the broad category fungal feeder/secondary decomposers: several
of these species can in principle compete for shared resources. We focused our
modelling and hypothesis testing on this assemblage.

3.3 Hypothesis testing

The RDA showed that PCNM-based spatial factors and environmental factors (PC1 and PC2 from PCA of environmental variables, see Fig. 1) could account for 31% of total community variation, the total effect of these factors being statistically significant at $p < 0.01$ following a permutational test. However, variance partitioning showed that 21% of this variation was attributable to spatial patterns in the environmental variables while 10% were accounted for by statistically significant (partial RDA, $p < 0.05$) spatial patterns not related to environmental variation. Less than 1% of variation was explainable in terms of environmental variation that was not spatially structured and this variation was not statistically significant. A RDA based just on environmental factors (i.e. implicitly including spatial structures) accounted for 22% of total variation, the effect of the environment being significant at $p < 0.01$. To test for the factor season, we extracted the residuals of the first, main RDA model and submitted these to a PERMANOVA test, which showed a significant effect of season ($F_{1, 78} = 4.17$, $p < 0.01$).

Introducing the season factor in the RDA increased total explained variation to 44%. A permutation test showed that the first five RDA axes were significant at $p < 0.01$ and these axes were therefore retained to define the niche space (i.e., based on spatial and temporal distance, which we, given our result, basically understand as the environmental or Grinnellian component of a species niche). A plot of the first two RDA axes (Fig. 2) and the main environmental gradients (based on PCA of environmental variables) showed that the first RDA axis is driven by a gradient in organic matter and soil structure. This gradient is associated with a certain species set while the second axis is driven by a second gradient due to the negative covariation of soil water and C:N. This second gradient is associated to a species set other than that associated to the first gradient. Size and the ^{15}N signature were negatively and significantly correlated with each other but scarcely correlated with the major

environmental gradients, although a positive and significant correlation was detected between ^{15}N and RDA1 (Fig. 3). After standardization, a Euclidean distance matrix was calculated from the Grinnellian niche space and correlated to the species trait distance matrix (based on ^{15}N , ^{13}C , size and depth distribution) via a Mantel test: no significant correlation was found (Fig. 4), which is inconsistent with the limiting similarity hypothesis.

None of the tested assemblages differed significantly from a neutral model for beta diversity (Supplementary Information, Fig. S3; whole assemblage, spring: $p = 0.10$; whole assemblage autumn: $p = 0.16$; fungal feeders spring: $p = 0.07$; fungal feeders autumn: $p = 0.10$, see Table S4 for the estimate of neutral model parameters). However, in all cases we observed assemblages with beta diversity higher than expected under neutrality (Fig. S3), and this trend was more pronounced in the fungal feeder group.

4. Discussion

4.1 Differences between environmental filtering and competition

In recent works investigating the role of deterministic and stochastic drivers of soil organism community structure (Beck et al., 2015; Caruso et al., 2012; Dumbrell et al., 2010; Gao et al., 2014; Lindo and Winchester, 2009; Nielsen et al., 2010) researchers contrasted environmental filtering, typically equated to niche dynamics, with spatial factors not dependent on patterns of environmental variation, sometimes called ‘pure’ spatial factors. These spatial factors are often understood as the effect of dispersal and/or demographic fluctuations in neutral assembly processes; but several ecologists, including those cited above, also recognise that these factors do not necessarily represent stochastic spatial factors (Anderson et al., 2011; Caruso et al., 2012; Smith and Lundholm, 2010). Besides the problem of the interpretation of spatial factors, a key but not often addressed aspect of this central topic is that environmental filtering may

imply competition for resources but does not necessarily imply resource-based niche partitioning dynamics: this is a point on which niche and neutral theorists may agree (HilleRisLambers et al., 2012; Hubbell, 2005), although from very different perspectives. At certain scales environmental filtering is compatible with neutral processes because in neutral dynamics competition for resources between species is not a driver of community structure while individuals, regardless of the species they belong to, must still exploit resources and fit their environment (Hubbell 2005). Different species can therefore come together into a local community if they are adapted to the environmental conditions of the locale, and in this sense the environment will tend to select for similar species (e.g., shade-tolerant species in shaded environments).. A neutrally assembled local community can therefore be environmentally filtered at certain scales while being neutral at scales at which competition among species has classically been postulated to structure communities (Etienne, 2007; Hubbell, 2005). It is in this general framework that we interpret our results: when biotic interactions start to be a fundamental driver and predictor of community structure neutral theories should be abandoned. Specifically, neutral theories directly contrast with resource-based niche partitioning processes. A first consideration is therefore that not all biological interactions should be considered, especially multitrophic interactions, which, apart from possible future developments, are usually outside the realm of application of neutral theories (Hubbell, 2005, 2001). For the first time, we have focused on a soil animal assemblage that was trophically defined by the use of stable isotopes of N and C. In doing so, we could start from the empirically validated assumption that competition for resources is a fairly valid possibility within the analysed assemblage. The small scale of the study also allowed us to assume that dispersal limitation, while still a possible factor given the size of our animals (Ettema and Wardle, 2002), should play a minor role. As shown by the analysis of the soil, communities were sampled along steep

environmental gradients in a very short distance. Accordingly, we observed a strong, spatially structured correlation between environmental gradients and the structure of the species assemblage. We can therefore conclude that the assemblage was subjected to environmental filtering. This result might imply that species living in different environmental patches spatially segregate to avoid competition locally. However, by no means can this result in itself be considered evidence of resource-based niche partitioning, which should also explain coexistence locally. This is an observational study: in order to reject non-neutral dynamics and find strong evidence of resource-based niche partitioning, we should have rejected neutral prediction of beta diversity and detected patterns consistent with the limiting similarity hypothesis along the environmental gradient, including the local scale of the assemblage inhabiting individual soil cores. Instead, neither could we reject neutral predictions of beta diversity nor could we find patterns consistent with the limiting similarity hypothesis. Observed beta diversity of the assemblage was higher than neutral predictions, as usually expected under environmental filtering (Caruso et al., 2012; Dornelas et al., 2006), but not significantly higher, with fairly high p-values in all cases but one. Species more similar in terms of spatial and seasonal distribution were not more dissimilar in terms of isotopic signature, size, and depth distribution. In theory, size could here be related to competition if we make the classical assumption that species at similar trophic positions avoid competition by differing in size: in this way competing species have access to similar resources in different places (i.e., colonization of differently sized soil pores; Weis-Fogh, 1948; Ritchie, 2009; Turnbull et al., 2014). The local community of our study is the cylindrical soil core used as sampling unit. In this relatively small locale, species that feed on similar resources and have similar size could still partition space by dwelling at different average depths but species weighed mean average depth was not a trait that could explain coexistence.

4.2 Niche partitioning mechanisms and competition

In spite of all the efforts we made to identify the possible dimensions along which competing species could partition their niches, none of these dimensions or their combination provided us with evidence of limiting similarities indicative of resource-based niche partitioning. In fact, the only pattern we have found is a slightly positive correlation between trophic position ($\delta^{15}\text{N}$ value) and the major environmental gradient along which the community is structured. However, the correlation seems made up by three low $\delta^{15}\text{N}$ values and one high $\delta^{15}\text{N}$ value, with the other points scattered in a fairly random manner. In any case, even if we accepted the validity of this correlation, this result would not support the limiting similarity hypothesis. We observed a significant fraction of spatial variation that was not related to environmental gradients. This variation can be due to stochastic but spatial factors such as dispersal, or it could be due to biotic interactions such as predation or competition. Predation can mediate competition by controlling the population of the more competitive species (Chase and Leibold, 2003): predators may spatially structure their prey but in the case of oribatid mites, and differently from collembolans, there is strong evidence that predation is not a strong factor controlling populations (Peschel et al., 2006). Competition and resource based niche partitioning could still play some role because we measured the traits that were most logically expected to be key traits for coexistence, but in fact we could have missed some important aspects. For example, there are limitations in the stable isotope markers we employed: the ^{13}C signature of animal fatty acids has now been demonstrated to be a finer marker for a detailed differentiation of fungal feeders (Pollierer et al., 2012; Ruess and Chamberlain, 2010) while with the method we employed we have been able to isolate a narrowly defined trophic assemblage (i.e. guild) but we might not have been able to differentiate trophic differences within this

434 assemblage. Natural variability in isotopic signatures may also suggest high
435 intraspecific variability in feeding strategies. This could be especially true for different
436 developmental stages. We are aware of data at this level for one species only (Schneider
437 et al. 2004) and these data suggest small differences between adults and nymphs but
438 other species could definitely vary their diet depending on developmental stage. The
439 interesting point is that high intraspecific variability can imply broad interspecific niche
440 overlaps at the species level, opening the way to neutral assembly processes. The same
441 arguments apply to temporal variation in species soil depth and may imply a theoretical
442 scenario for which levels of competition vary in space (both horizontally and vertically)
443 and time as a function of fluctuations in population densities.

444 Another limit of our study is that we might not have included all the species relevant to
445 the analysed assemblage. We focused on fungal feeder/secondary decomposer oribatid
446 mites, which is by far the most diverse and abundant group of microarthropods together
447 with collembolans. However, there are other fungal feeders/secondary decomposers in
448 soil, for example collembolan species. We cannot exclude that competition for
449 resources would have been a strong driver of an assemblage that included all the species
450 competing for a limited set of resources.

451 Finally, our multivariate analysis suggested that seasonal variation is potentially a key
452 niche dimension although our study is deficient in terms of temporal replication.
453 Species competing for similar resources could peak at different times of the year to
454 avoid competition, basically for the same principle for which competing species may
455 segregate spatially. Nevertheless, only future studies will tell whether the observed
456 temporal patterns depend on a temporal form of environmental filtering (e.g.
457 seasonality) or resource based niche partitioning mediated by temporal fluctuations in
458 resources and population densities, or both.

Overall, our results indicate that environmental filtering and resource-based niche partitioning can be decoupled in soil animal assemblages while the burden of the proof of resource-based niche partitioning in soil community still remains with the ecologist.

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Data Accessibility

All data (species abundances, environmental and geographical data, isotopic data, trait data) are uploaded as Supporting Information

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Fig. Legends

Fig. 1 Principal Component Analysis (PCA) of the correlation matrix (z-scores) of environmental variables: 77% of total variance can be summarized in the first two axes. PC1 (53%) described a main gradient in organic matter (organic C and total N) and soil structure (Mean Weight Diameter, MWD); PC2 (24%) described a negative covariation between water content and the C:N ratio. The vectors associated with the variables are based on PCA eigenvectors (i.e. variables loadings on PCA axes).

Fig. 2 First two RDA axes based on a model including spatial vectors, environmental gradient and seasons. Only species points are displayed to show which species are associated with the two environmental gradients. See Table 1 for species labels. This RDA model accounted for 44% of total species matrix. The RDA axis 1 is driven by a gradient of organic matter and soil structure (PC1 of Fig. 1). RDA axis 2 by a contrast between water content and C:N ratio (PC2 of Fig. 1);

Fig. 3 a) correlation between size (x-axis) and species trophic position (^{15}N , y-axis) is negative and statistically significant; b and c), correlation between species scores of RDA 1 (y-axis; see Fig. 2) and size (panel b) or ^{15}N (panel c), on the x-axis. RDA1 is a proxy for the environmental, spatial and temporal (seasonality in this case) components of niche. No or weak correlation is observed in panel c and d respectively. Similar figures were drawn (but now shown here) for the first five RDA axes, with the same result. Each data point represents a species.

Fig. 4 Niche distance between species is based on the species scores of the statistically significant axes of an RDA (spatial vectors, seasons, and environmental variables). The Euclidean distance between any two species in the vectorial space defined by RDA axes

669 reflects predicted spatial, temporal and environmental distances: the further apart any
670 two species are in this space the further apart these species are in terms of their niche.
671 This RDA-based Euclidean distance matrix was correlated to the species trait distance
672 matrix (based on ^{15}N , ^{13}C , size and depth distribution) via a Mantel test: the Fig. and test
673 show a remarkable lack of correlation, which is inconsistent with the limiting similarity
674 hypothesis.